



**IVD** For *in Vitro* Diagnostic Use

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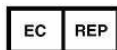
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# AmpliSens® *Vibrio cholerae* -FRT

PCR kit

## Instruction Manual

# AmpliSens®



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## 1. INTENDED USE.

**AmpliSens® *Vibrio cholerae*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and identification of *Vibrio cholerae* DNA in biological material and environmental compartments by using real-time hybridization-fluorescence detection.

## 2. PRINCIPLE OF PCR DETECTION.

*Vibrio cholerae* DNA (if Hly sequence is present) and identification of pathogen *Vibrio cholerae* strains (if main virulence factors – CtxA, tcpA – are present), belonging to serogroups O1 (if amplification target wbeT present) or O139 (if amplification target wbf present) by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Vibrio cholerae* strains primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Vibrio cholerae*-FRT** PCR kit is a qualitative test, which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. PCR analysis is carried out in multiplex format in two tubes: “Screen” – the amplification of CtxA target (FAM/Green), tcpA target (ROX/Orange) and IC target (JOE/Yellow/HEX), “Type” - the amplification of Hly target (JOE/Yellow/HEX) - cholera germs of all groups, wbeT (FAM/Green) - belonging to serogroup O1, wbf (ROX/Orange) - belonging to serogroup O139. It's necessary to carry out both “Screen” and “Type” reactions for results interpretation. **AmpliSens® *Vibrio cholerae*-FRT** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. Wax melting and reaction mix components occur only at 95 °C.

## 3. CONTENT.

**AmpliSens® *Vibrio cholerae*-FRT** PCR kit is produced in 1 form:

AmpliSens® *Vibrio cholerae*-FRT PCR kit variant FRT (for use with RG) **REF** R-B53(RG)-CE.

**AmpliSens® *Vibrio cholerae*-FRT** PCR kit, variant FRT includes:

| Reagent  | Description            | Volume (ml) | Quantity           |
|--|------------------------|-------------|--------------------|
| <b>PCR-mix-1-FEP/FRT <i>Vibrio cholerae</i> screen</b><br>ready-to-use single-dose test tubes ( <i>under wax</i> ) | colorless clear liquid | 0.008       | 55 tubes of 0.2 ml |
| <b>PCR-mix-1-FEP/FRT <i>Vibrio cholerae</i> type</b><br>ready-to-use single-dose test tubes ( <i>under wax</i> )   | colorless clear liquid | 0.008       | 55 tubes of 0.2 ml |
| <b>PCR-mix-2-FL</b>  | colorless clear liquid | 0.77        | 1 tube             |
| <b>Positive Control DNA <i>Vibrio cholerae</i> screen (C+<sub>screen</sub>)</b>                                    | colorless clear liquid | 0.1         | 1 tube             |
| <b>Positive Control DNA <i>Vibrio cholerae</i> type (C+<sub>type</sub>)</b>  | colorless clear liquid | 0.1         | 1 tube             |
| <b>Positive Control IC</b>   | colorless clear liquid | 0.1         | 1 tube             |
| <b>DNA-buffer</b>  | colorless clear liquid | 0.5         | 1 tube             |
| <b>Negative Control (C-)*</b>  | colorless clear liquid | 1.6         | 2 tubes            |
| <b>Internal Control <i>Vibrio Cholerae</i> (IC)**</b>  | colorless clear liquid | 0.5         | 1 tube             |

\* must be used in the isolation procedure as Negative Control of Extraction.

\*\* add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (DNA-sorb-B, **REF** K1-2-50-CE).

AmpliSens® *Vibrio cholerae*-FEP PCR kit is intended for 55 reactions, including controls.

## 4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barriers (up to 200 µl)
- Tube racks
- Vortex mixer
- PCR box
- Personal thermocyclers (for example, «Rotor-Gene» 3000 or 6000 (Corbett Research, Australia) or equivalent)
- Disposable polypropylene microtubes for PCR with 0.2 ml capacity (for example, “Axygen”, USA)

- Refrigerator for temperature between 2 and 8 °C
- Deep-freezer with temperature not more than minus16°C
- Waste bin for used tips
- Agents kit for work space treatment

## 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

## 6. SAMPLING AND HANDLING.



Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® Vibrio cholerae-FRT-PCR kit is intended for the analysis of DNA extracted by using DNA isolation kit from biological material and environmental compartments.

### The following material is used for analysis:

#### **Clinical material samples:**

- 1,0 – 2,0 g (or 1-2 ml in case of diarrhea) of faeces, native or transferred into tube with 5 ml of 1% peptone water are used after preliminary preparation;
- 1-2 ml of vomit masses, native or transferred into 5 ml of peptone water are used after preliminary preparation;
- rectum wall scraping from depth of 5-6 cm, taken by dry sterile probe (probe's working part with tampon is to be placed into 1.5 ml tube with 0.5 ml of 1% peptone water, the rest of the probe is to be broken and deleted). 50 µl of solution is used for analysis.

#### **Autopsy material samples:**

- upper, medial and lower sections small intestine content is transferred into tube with 5 ml of 1% peptone water are used after preliminary preparation.

#### **Environmental samples (for monitoring aim):**

- water (from water body, wastewater, drinking water) is sampled and treated in compliance with local authorities requirements. First peptone water (after preliminary preparation) is used for analysis;
- silt and hydrobionts are sampled and treated in compliance with local authorities requirements. First peptone water (after preliminary preparation) is used for analysis.

#### **Environmental samples (nidus of infection):**

- water (from water body, wastewater, drinking water) is sampled and treated in compliance with local authorities requirements. Then it is preliminary filtered out through the filters with pore diameter – 8 µm (or paper filters) and finally filtered out by using of filters with pore diameter – 0.45 µm. These filters are placed into the tubes with 3 ml of physiological solution and boiled during 10 min. 50 µl of solution is used for analysis. In case of negative

analysis result it's needed to make the inoculation of washouts from filters in compliance with local authorities requirements and to test the first peptone water (after preliminary preparation).

- washouts from the surfaces of object (10 x 10 cm area), sampled by sterile probe, which was wetted by physiological solution (probe's working part with tampon is to be placed into 1.5 ml tube with 0.5 ml of 1% peptone water, the rest of the probe is to be broken and deleted). 50 µl of solution without preliminary preparation is used for analysis.

**Food products:** are sampled and treated in compliance with local authorities requirements. First peptone water (after preliminary preparation) is used for analysis.

**Germ cultures,** suspected in *Vibrio cholerae* presence:

- colony is to be resuspended in 0,5 ml of physiological solution or phosphate buffer. 50 µl of suspension is used for analysis.

Studied material's transportation is strictly carried out in compliance with local authorities requirements.

#### **Material's preliminary preparation**

Any operation with studied material transportation is carried out in compliance with local authorities requirements.

All manipulations, connected with probes preparation, are carried out by varying volume pipettors with using of disposable polypropylene microtubes of 1.5 ml or 10.0 ml volume and tips with aerosol barriers. Disposable plastic dishes (tubes, tips) are to be thrown into the special container with suitable disinfectant. They are to be utilized in compliance with local authorities requirements.

#### **Native faeces:**

- 10-20 % faeces suspension preparation (watery faeces are used without suspension preparation).
  1. 4 ml of saline or phosphate buffer is to be transferred into 5 ml volume tubes with tightly closed cap.
  2. 0.5 – 1.0 g (near 1-2 ml) faeces are transferred into tubes. Use an individual tip with aerosol barriers (or disposable spatula) for each tube. The content of the tube is to be stirred carefully to form the homogeneous suspension.
- B. Faeces bacterial fraction preparation:

From tubes with faecal suspension 1 ml is to be transferred to 1.5 ml tube with tightly closed cap then it's to be centrifuged during 5 min at 12000 rpm. For DNA isolation 50 µl of light fraction from the board of transparent liquid and solid faecal fractions is to be used.

#### **Faeces or vomit masses, placed into 1 % peptone water:**

- A. The content of the tube is to be stirred carefully to form the homogeneous suspension.
- B. Bacterial fraction preparation:

1 ml of suspension is to be transferred to 1.5 ml tube with tightly closed cap then it's to be centrifuged during 5 min at 12000 rpm. For DNA isolation 50 µl of light fraction from the board of transparent liquid and solid faecal fractions is to be used.

#### **Autopsy material samples (small intestine content):**

The content of the tube is to be stirred carefully to form the homogeneous suspension. For DNA isolation 50 µl of suspension is to be used.

#### **Liquid culture (peptone water after bacterial inoculation):**

- from the surface of peptone water 1.2 – 1.4 ml is sampled into the tube of 1.5 ml volume then it is to be centrifuged during 10 min at maximum revolutions (10-12 thousand rpm). Supernatant is to be deleted. Precipitate is to be resuspended in 300 µl of saline or phosphate buffer. 50 µl of solution is used for analyses.

## **7. PROTOCOL.**

### **7.1. DNA Isolation**

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-B", **REF** K1-2-50-CE.



Carry the DNA isolation according to the manufacturer's instructions.



Prepare the required number of disposable tubes including the negative control of isolation.



50 µl of Negative Control and 50 µl of sample are transferred into the tubes with **Lysis Solution** and **Internal Control *Vibrio Cholerae*** by using of tips with aerosol barriers

### **7.2. Preparing the PCR.**

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

### 7.2.1 Preparing tubes for PCR.

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Vibrio cholerae* screen** and **PCR-mix-1-FEP/FRT *Vibrio cholerae* type** for amplification of DNA from clinical and control samples. Mark tubes by «S» and «T»
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP/FRT**
3. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage into prepared tubes.



It's necessary to avoid sorbent getting into reaction mix.

4. Carry out the control amplification reactions:

- NCA** - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C<sup>+</sup>screen** - Add **10 µl** of **Positive Control DNA *Vibrio cholerae* screen (C<sup>+</sup>screen)** to the tube with **PCR-mix-1-FEP/FRT *Vibrio cholerae* screen** labeled **C<sup>+</sup>screen** (Positive Control of Amplification).
- C<sup>+</sup>type** - Add **10 µl** of **Positive Control DNA *Vibrio cholerae* type (C<sup>+</sup>type)** to the tube with **PCR-mix-1-FEP/FRT *Vibrio cholerae* type** labeled **C<sup>+</sup>type** (Positive Control of Amplification).
- IC+** - Add **10 µl** of **Positive Control IC** to the tube with **PCR-mix-1-FEP/FRT *Vibrio cholerae* screen** labeled **IC+** (Positive Control of Amplification).

### 7.2.2. Amplification

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

**RG program**

| Step      | Temperature, °C | Time   | Fluorescence detection            | Cycle repeats |
|-----------|-----------------|--------|-----------------------------------|---------------|
| Hold      | 95              | 5 min  | –                                 | 1             |
| Cycling   | 95              | 10 sec | –                                 | 10            |
|           | 60              | 25 sec | –                                 |               |
|           | 72              | 10 sec | –                                 |               |
| Cycling 2 | 95              | 10 sec | –                                 | 35            |
|           | 56              | 25 sec | FAM/Green, JOE/Yellow, ROX/Orange |               |
|           | 72              | 10 sec | –                                 |               |

3. Fluorescence detection is on the 2-nd pass (**56°C**) in FAM/Green, JOE/Yellow and ROX/Orange fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

### 8. DATA ANALYSIS.

The signal is considered to be positive, if the corresponding fluorescence accumulation curve crosses threshold line. Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

#### Results interpretation.

**Results for controls for test with PCR-mix-1-FEP/FRT *Vibrio cholerae* screen**

| Control               | Controlled stage | Results           |                 |                   | Interpretation |
|-----------------------|------------------|-------------------|-----------------|-------------------|----------------|
|                       |                  | FAM/ Green (CtxA) | JOE/Yellow (IC) | ROX/Orange (tcpA) |                |
| C-                    | DNA isolation    | Neg               | Pos (< Z*)      | Neg               | OK             |
| NCA                   | Amplification    | Neg               | Neg             | Neg               | OK             |
| C <sup>+</sup> screen | Amplification    | Pos (< Y*)        | Neg             | Pos (< X*)        | OK             |
| IC+                   | Amplification    | Neg               | Pos (< N*)      | Neg               | OK             |

**Results for controls for test with PCR-mix-1-FEP/FRT *Vibrio cholerae* type**

| Control             | Controlled stage | Results         |                         |                   | Interpretation |
|---------------------|------------------|-----------------|-------------------------|-------------------|----------------|
|                     |                  | FAM/ Green (O1) | JOE/Yellow (V.cholerae) | ROX/Orange (O139) |                |
| C-                  | DNA isolation    | Neg             | Neg                     | Neg               | OK             |
| NCA                 | Amplification    | Neg             | Neg                     | Neg               | OK             |
| C <sup>+</sup> type | Amplification    | Pos (< M*)      | Pos (< K*)              | Pos (< L*)        | OK             |

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

1. The sample is considered to be positive for required target if its Ct value is defined in the results grid in FAM/Green channel and if it does not exceed Y, X, M, K or L
2. The sample is considered to be negative for required target if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line).

### Analysis results' estimating

| Variants   | PCR-mix-1-FEP/FRT <i>Vibrio cholerae</i> screen |                          |                   | PCR-mix-1-FEP/FRT <i>Vibrio cholerae</i> type |                         |                   |
|--|---|--------------------------|-------------------|---|-------------------------|-------------------|
|  | Ct value on channel                             |                          |                   |   |                         |                   |
|  | FAM/Green (CtA)                                 | JOE/Yellow (IC)          | ROX/Orange (tcpA) | FAM/Green (O1)                                | JOE/Yellow (V.cholerae) | ROX/Orange (O139) |
| V.cholerae O1 toxigenic  | Pos (< Y*)                                      | Any value or its absence | Pos (< X*)        | Pos (< M*)                                    | Pos (< K*)              | Neg               |
| V.cholerae O139 toxigenic                                      | Pos (< Y*)                                      | Any value or its absence | Pos (< X*)        | Neg   | Pos (< K*)              | Pos (< L*)        |
| V.cholerae O1 NON toxigenic, but contained the sequence tcpA   | Neg   | Pos (< Z*)               | Pos (< X*)        | Pos (< M*)                                    | Pos (< K*)              | Neg               |
| V.cholerae O139 NON toxigenic, but contained the sequence tcpA | Neg   | Pos (< Z*)               | Pos (< X*)        | Neg   | Pos (< K*)              | Pos (< L*)        |
| V.cholerae O1 NON toxigenic                                    | Neg   | Pos (< Z*)               | Neg               | Pos (< M*)                                    | Pos (< K*)              | Neg               |
| V.cholerae O139 NON toxigenic                                  | Neg   | Pos (< Z*)               | Neg               | Neg   | Pos (< K*)              | Pos (< L*)        |
| V.cholerae not O1 and not O139                                 | Neg   | Pos (< Z*)               | Neg               | Neg   | Pos (< K*)              | Neg               |
| Comma bacillus are not detected                                | Neg   | Pos (< Z*)               | Neg               | Neg   | Neg                     | Neg               |

\*For X, Y, Z, N, M, K, L values see Appendix 1.

### 9. TROUBLESHOOTING.

1. If the Ct values are absent on FAM/Green and ROX/Orange channels and the Ct value on JOE/Yellow channel is also absent or exceeds Z when using of PCR-mix-1-FEP/FRT *Vibrio cholerae* screen then the PCR analysis and DNA isolation should be repeated.
2. If the positive signal on any target except Hly (negative result on JOE/Yellow channel when using of PCR-mix-1-FEP/FRT *Vibrio cholerae* type and the Ct value does not exceed Z on JOE/Yellow channel when using of PCR-mix-1-FEP/FRT *Vibrio cholerae* screen) is obtained then the result sample is considered to be invalid. The sampling and the analysis should be repeated.
3. If the Ct value on JOE/Yellow channel is absent when using of PCR-mix-1-FEP/FRT *Vibrio cholerae* type and the conditions of item 1 are satisfied then the analysis should be repeated from the stage of DNA extraction.
4. The absence of positive signal in a sample with positive control could indicate that the amplification program is chosen in correctly or it could indicate of other mistakes made during PCR run. In this case the PCR analysis should be repeated.
5. Positive signal for negative control (C-) on FAM/Green and/or ROX/Orange and for Negative Control of Amplification (NCA, DNA-buffer) on any channel indicates the reagent or sample

contamination. In such case the analysis results are considered to be invalid. The analyses should be repeated and measures to detect and eliminate the contamination source are to be taken.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

### 10. STABILITY AND STORAGE.

All components of the **AmpliSens® Vibrio cholerae-FRT** are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® Vibrio cholerae-FRT PCR kit** are to be stable until labeled expiration date.



All components of the **AmpliSens® Vibrio cholerae-FRT** are to be stored away from light.

### 11. SPECIFICATIONS.

#### 11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® Vibrio cholerae-FRT** PCR kit is not less than 1x10<sup>3</sup> copies per 1 ml of sample (c/ml).



The claimed analytical features of **AmpliSens® Vibrio cholerae-FRT** PCR kit are guaranteed only when additional reagents kit "DNA-sorb-B" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) is used.

#### 11.2. Specificity.

Specificity of **AmpliSens® Vibrio cholerae-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® Vibrio cholerae-FRT** PCR kit was confirmed in laboratory clinical trials.












### 12. REFERENCES.

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

### 13. QUALITY CONTROL.

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **AmpliSens® Vibrio cholerae-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

### 14. EXPLANATION OF SYMBOLS.

|   |                                    |  |  |
|---|------------------------------------|--|--|
|    | Manufacturer                       |   | Temperature limitation                               |
|    | Use by                             |   | Batch code   |
|    | For <i>in Vitro</i> Diagnostic Use |   | Version  |
|    | Catalogue number                   |   | Authorised representative in the European Community. |
|   | Contains sufficient for <n> tests  |  | Caution, consult accompanying documents              |
|  | Consult instructions for use       | <b>NCA</b>   | Negative Control of Amplification                    |
| <b>C-</b>   | Negative control of Extraction     | <b>C+</b>  | Positive control of Extraction                       |
| <b>IC</b>   | Internal control                   |  |  |